Molecular-genetic identification of porcine enteroviruses

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Porcine diseases caused by enteroviruses are known since 1930, when Trefny has first diagnosed enzootic porcine encephalomyelitis near Tezen in Czechoslovakia, and Klobauk described this disease in 1933; later, the disease has acquired several other names, including porcine poliomyelitis, Teschen disease, Klobauk disease, Talfan disease, Teschen's disease etc. Studies performed by Klobauk have revealed that Teschen disease is caused by a neurotropic virus, pathogenic exclusively for pigs.

Successful use of cell cultures for poliovirus reproduction by Enders, Weller, and Robbins (1949) has enabled isolation of multiple previously unknown virus species from different organs of humans and animals, including pigs.

Isolation and study of viruses – agents of enterovirus diseases of animals was initiated in mid-1960th, and the first enteroviruses isolated from animals were porcine enteroviruses.

Porcine enteroviruses were first isolated in 1956 by Moskovici, Ginervi, and Mazzarachio, and in 1958 Beran, Werder, Wenner have reported isolation of 376 cytopathic agents from 1-10-day piglets in one of examined farms in US.

The same year (1958), Larski, Szatlarski, Szurman have reported isolation of two strains of enzootic porcine encephalomyelitis in Poland.

Yet, more expanded study of porcine enteroviruses was initiated following publication of reports by Lamont, Betts (1958), and Webster (1959) concerning isolation of viruses from pigs with clinical signs of enteritis. In later publications, Betts, Jennings (1960), Betts, Jennings, Kelly (1960), Betts (1960) have reported cell culture isolation of two serologically related strains of polincephalomyelitis virus, T 80 и T 52A, from tonsils of a "healthy" piglet, which caused poliencephalomyelitis when used in different methods of inoculation of piglets devoid of colostrum.

Sibalin (1963) has isolated intestinal I-6 virus in the foci of the disease accompanied with paresis and paralysis. The isolated virus was identical by antigenic composition to Talfan disease agent.

Isolation of enteroviruses from pigs in Hungary was reported by Szent-Jvanyi (1963), who has isolated 505 enterovirus strains from 1585 rectal swab samples collected from piglets of different age groups.

Veznikova, Gols, Gerny (1969) have isolated 13 enterovirus strains from lungs of pigs suffering from pneumonia.

Since 1966, great attention to the study of porcine enteroviruses was paid by Derbyshire with several co-authors. Knowles et al. (1979, 1980) have reported isolation in Great Britain and identification of three novel serotypes of porcine enteroviruses.

Since 1969 till nowadays, we (Romanenko V., 1977; Romanenko V. et al., 1972, 1973, 1974, 1994, 1996, 1998, 2007, 2009) have carried out isolation and detailed study of porcine enteroviruses, isolated in different regions of former USSR from pigs of different age groups. Studying enteroviruses, isolated from pigs, we were guided by Pette (1966), who had proposed limiting ECSO viruses by the agents of viral nature, which:

1) possess special affinity to gastrointestinal tract, where their primary reproduction occurs;

2) exert pronounced cytopathic effect in cell culture of porcine kidneys;

3) are characterized by virus particle size not exceeding 30 nm;

4) show resistance to chloroform;

5) are stable within wide pH range;

6) are of relative thermal stability, with their thermal stability increasing following treatment with Mg^{++} and Ca^{++} cations;

7) are resistant against trypsin;

8) contain RNA.

During the first stages, isolation of porcine enteroviruses was carried out from primary-trypsinized cells of pig fetal kidney (PPK). Cytopathic effect in this

culture was rather pronounced. Nevertheless, the character of cytopathic changes, caused by porcine enteroviruses in cell cultures, was not similar for all the viral strains.

Thus, according to Betts (1960), Jennings, Kelly, Betts (1962), inoculation of cell cultures with enterovirus strains T80 and T 52A first resulted in swelling of affected cells. Some of the cells became roundish during that stage. Later, majority of the cells became affected, rounding of the cells became more pronounced, and the rounded cells started exfoliating from the glass.

Enterovirus isolated by Lamont and Betts (1960) (V13 strain) caused cell accumulation and nucleolus swelling in affected cells to a lesser degree.

Studying over 200 viral isolates, isolated from porcine faeces, Zoletto (1964, 1965) divided them into three types:

I type – formation of roundish cells of high light refraction, grouped in the center of the culture;

II type – appearance of cells with high refractive index, with characteristic cytoplasm evagination, in the infected monolayer;

III type – appearance of disseminated roundish, light refracting cells in the infected monolayer.

Kadoi, Kabori, Morimoto (1970) have divided the studied porcine enteroviruses into two types according to the nature of CPE (cytopathic effect). Enterovirus strains of the first type caused CPE characterized by nuclear hyperchromatism, crumpled cytoplasm, rounding, and aggregation, followed by destruction of affected cells. Cell cultures infected with the strains causing degeneration of type II had pyknotic nucleus, crumpled cytoplasm with evagination, and underwent granulation and disintegration.

Poliomyelitis virus is the first virus with detected ability for propagation in tissue culture of a human embryo and promotion of cytopathic effect in it; this property was later found to be characteristic of enteroviruses (Enders, Weller, Robbins, 1949). This effect consists in rounding and shrinkage of cells,

compaction of their nuclei and disturbance of monolayer integrity due to cell tearing from the glass surface.

Similar changes are detected in case of ECHO virus infection. Yet, the cytopathic effect caused by ECHO 22 and 23 viruses differs from the described above due to marked changes in the nucleus at late stages of the infection: nucleoli disintegration and chromatin accumulation near the nucleus membrane. Infection of Coxsackie B1 virus results in cell hypertrophy and parietal location of nucleoli. Later, granular or filamentous segregation of basic nucleus components occurs (Garali et al., 1974).

Pleva, Mesaros (1968) cultivated PPK in cell cultures and studied CPE character of 17 enterovirus strains they have isolated from pigs and 8 typical strains obtained from different places. The authors used both primary cultures of pig fetal kidneys and transferred PK line in these experiments. CPE character was similar in cells of both types. Viral CPE manifested itself in 24 hours following infection and was characterized by rounding of the cells and thickening of cell walls. Sometimes, the changes of another type were observed: the cell wall interfaces became indistinct, and the cytoplasm penetrated into intercellular space in the form of drops. In this case, the cellular surface gained serrated outline. By the end of the 3-rd day, complete cell degeneration and cell detachment from the glass occurred.

Both CPE types were observable simultaneously in the same cell culture infected with the same viral strain. The authors find classification of isolated porcine enteroviruses by CPE type impossible. We have obtained similar data (V. Romanenko, 1977).

In our experiments, CPE character of enteroviruses at inoculative dose 1000-10000 viral particles was of similar type, irrespective of virus strain and species, in PPK cell cultures and transferable cell culture of pig fetal kidney (TPFK). Cytopathic effect occurred in 16-20 hours following infection, and for certain strains − in 36-40 hours following infection, and was characterized by appearance of roundish cell foci; up to 50-70% of the monolayer cells were affected in another 4-6 hours. The roundish cells were granular and had increased optical density. CPE

caused by all the studied strains terminated almost simultaneously by complete destruction of the cell culture monolayer.

When the high viral doses (10^6-10^7 TCD_{50}) (tissue cytopathogenic dose causing the death of 50% of monolayer cells) were used for inoculation of TPFK cell cultures, CPE manifested itself through cellular fusion: cytoplasms of the affected cells were fused with each other, forming symplasts. Following the use of viral dose up to 10^5 TCD_{50} for cell culture inoculation in following passages, viral CPE manifested itself through rounding of the affected cells.

The data presented demonstrate that enterovirus classification by CPE type is ungrounded, which has been proven by the experiments of Pleva and Mesaros (1968); considering the obtained data, these authors find classification of the studied viruses by CPE type impossible. Multiple studies we have performed (V. Romanenko et al., 1969-2009), as well as experimental data obtained with human enteroviruses (Enders et al., 1949, Garali et al., 1974), confirm the opinion of Pleva and Mesaros.

The first classification of porcine enteroviruses was developed by Betts (1962), who initially divided enteroviruses into 9 antigenic serogroups, and later Alexander, Betts (1967) classified porcine enteroviruses they have studied into 10 serological groups using cross-neutralization reaction.

In 1971-1972, Dunne H.W., Wang I.T. and Ammerman E.H of Pirbright Laboratory carried out a comparative study of porcine enterovirus strains isolated in different countries: 13 strains from England, 5 stains from Japan, 13 strains from Canada, 3 stains from California, 6 stains from Maryland and 32 from Pennsylvania, which were finally classified into eight serogroups:

serogroups	–	strains
1-st	–	Teschen, Talfan, F 65(SMEDIC), PS 35 etc.
2-nd	–	T80, T52A, F59, F17, J2, T3
3-rd	–	F34, 02b (SMEDIB), PE1, PE3
4-th	–	F78,PS 15-PS 20,PS 36,PS 38, DE 8
5-th	–	F12, J3
6-th	–	F7,PS 37 (SMEDIE), T4, J5, ECPO-2

7-th	−	F43, WR 2
8-th	−	V13, PS 27 (SMEDIA),PS 23, PS 26, PS 28-PS 30, A1, ECPO-1, ECPO-5 PE 4, PE 5, PE 7.

This classification is used for studies and ordination of enteroviruses isolated from pigs, including our studies.

The collection of strains belonging to 8 serotypes (Teschen, Talfan, T80, F59, F34, F78, F12, F7, F43, V13), obtained from Dr. Derbyshire J.B. (Pirbright Laboratory, England) in 1970, presented the basis for study and classification of porcine enterovirus strains we have isolated within the territory of Ukraine, Russian Federation and Moldova.

Following study of reproductive properties of 850 virus isolates from bowels of patients with clinical signs of gastroenteritis, pneumonia, and encephalomyelitis, as well as from pigs which have contacted with the mentioned patients, we have selected 100 strains, which, in parallel with the strains obtained from Pirbright Laboratory, were used for study of reproductive properties, resistance to chloroform, ether, trypsin, and media with different pH values; thermal stability; effect of nucleic metabolism inhibitors on reproduction of porcine enteroviruses in cell cultures; ability for plaque formation and plaque type; viral particle morphology, and antigenic characteristics.

Finally, the studies viruses were classified as belonging to Enterovirus genus, Enterovirus suis sp. At the same time, properties of porcine enterovirus strains we have isolated corresponded completely to generalized data by Pette (1966) as to biological, physicochemical and genetic characteristics of porcine enteroviruses, and also corresponded to the properties of human enteroviruses (M.K. Voroshilova, 1979).

Study of antigenic characteristics of isolated enterovirus strains using the collection of reference strains by Laboratory Pirbright as the basis has enabled us to reveal 14 novel previously unknown porcine enterovirus serotypes, which reference strains are protected by author's certificates of former USSR. Our

enterovirus collection, including reference strains of 1-8 serotypes obtained from Laboratory Pirbright and 14 reference strains we have detected, consists of 23 serotypes presented in Table 1.

Table 1. Reference strains of porcine enteroviruses

Serotype	Reference strains	Serotype	Reference strains
1	Teschen, Talfan	13	Л-90
2	T 80, T-52A, F 59	14	М-116
3	F 34	15	Ч-73
4	F 78	16	Г-95
5	F 12	17	Б-111
6	F 7	18	Ч-184
7	F 43	19	Д-229
8	V 13	20	И-249
10	M 2323	21	П-142
11	К-9	22	В-151
12	К-22	23	И-393

Rasmussen (1969) has classified enteroviruses isolated in Denmark into 10 serotypes. Christov (1966) attributed enteroviruses isolated in Bulgaria to three serotypes. We have found no further information concerning classification of porcine enteroviruses by other investigators.

In 1979, Knowles N.J.; Buckley L.S. and Pereira H.D. supplemented the collection of porcine enteroviruses with three new serotypes, included to the collection of Pirbright Laboratory as enterovirus serotype 9, 10 and 11. In 1990, Honda, Kimata, Hattori, Kumagai, Tsuda, Tokui, and Auerbach, Witte in 1993 revealed several other novel enterovirus serotypes, which were studied only in virus neutralization reaction and thus were not included to the collection of porcine enteroviruses of Laboratory Pirbright (Materials on laboratory identification of porcine enteroviruses, presented by Knowles N.J in 1994 in International Symposium on Porcine Picornavirus Infections, Greitswald, Germany, 2-3 May 1994).

In 1997, a report by Knowles N.J. entitled „Molecular Identification of Porcine Enteroviruses with the Question “Are Teschen-like Viruses True

Enteroviruses?" appeared in Internet. Here, Knowles N.J. reports genome sequencing of porcine enteroviruses of 8-th, 9-th, and 10-th serotypes he had classified to II-nd and III-rd groups by CPE type. The sequencing results have led him to the conclusion of their relationship with human and animal enteroviruses. Nevertheless, the representatives of other serogroups of porcine enterovirus collection Laboratory Pirbright, classified by him to the I-st group according to CPE type (1-7 и 11-13), are not typical enteroviruses for the following reasons:

- thermal stability: group I viruses are not inactivated during heating at 50^0C for 1 hour, like true enteroviruses.
- Ion effect: usually, the presence of 1M $MgCl_2$ protects enteroviruses from inactivation at 50^0C. Group I viruses are still inactivated during heating at 50^0C in the presence of halogen ions. Here, they show similarity to Encephalomyocarditis (EMCV) virus, inactivated during heating in the presence of halogen ions in certain pH interval.
- Formaldehyde sensitivity. 0.1% formaldehyde treatment results in destruction of capsid structure of group I viruses, but not group II and III viruses.
- Electron microscopy reveals inflation of viral segments.
- Growth properties, CPE type: the time necessary for CPE appearance at inoculation dose 100 TCD_{50} is 4-5 days. This is a lower value than that of most enteroviruses. CPE characteristics of group I are distinct from those of group II and III.

Thus, interpreting his data, Knowles N.J. (1997) remarked that viruses belonging to CPE I group (1-7 and 11-13) can hardly be classified as Enterovirus genus. He has attributed the isolated viruses to a new genus, Teschovirus.

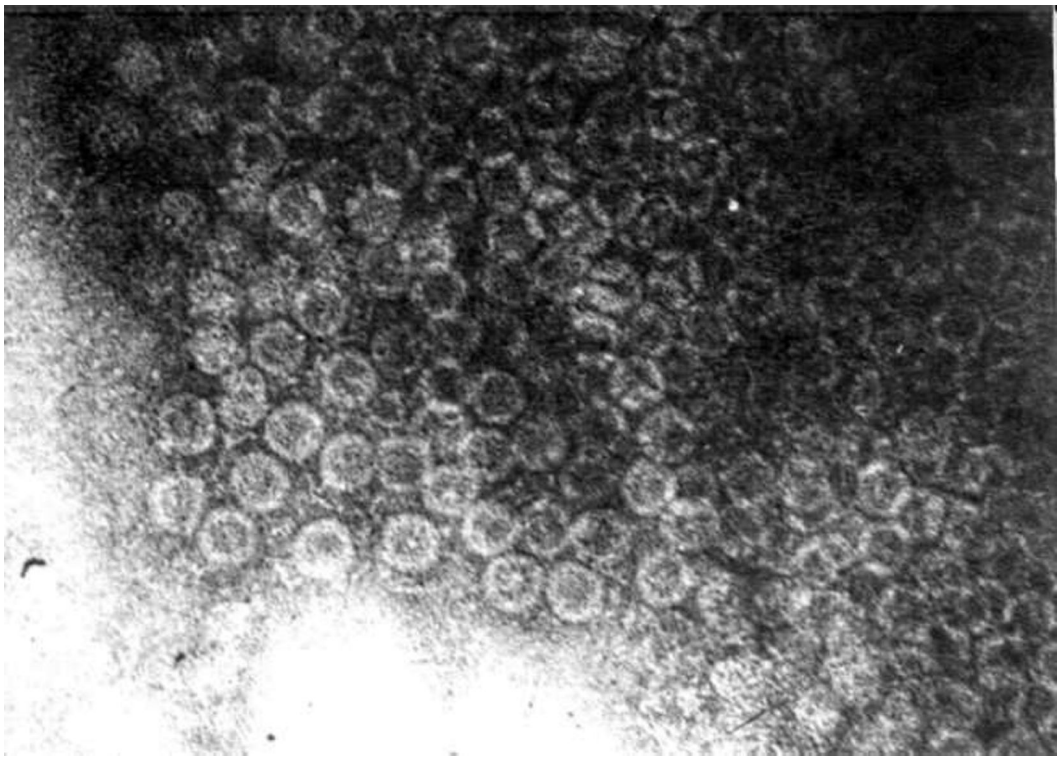
This proposition by N.J. Knowles has been ratified in 1999 by mail drawing of voting members of Executive Committee on Taxonomy of Viruses (ICTV), and approved in the same 1999 by XI International Congress of Virology by the resolution: „To rename porcine enteroviruses of group I into porcine teschoviruses and to distinguish them into a new genus Teschovirus, approved as the 9-th genus in Picornaviridae family" (CR Pringle, 1999) .

Does the mentioned distinction of porcine enteroviruses into 2 genera, Enterovirus and Teschovirus, reflect the reality?

According to modern classification, elaborated by International Committee of Viral Nomenclature, porcine enteroviruses belong to Enterovirus genus and should possess certain biological, physicochemical, morphological and genetic properties, while the materials by N.J. Knowles, presented to ICTV as those determining a new virus genus in Picornaviridae family, are insufficient for distinction of a new genus, as the data on biological and genetic properties are absent, and the characteristics presented by N.J. Knowles for establishment of a new genus do not correspond to the requirements determining species belonging of the viruses. The properties N.J. Knowles presented to ICTV for establishment of a new virus genus included only certain physicochemical characteristics of viruses, which, besides, were different from our data and data of other investigators; the data concerning classification of porcine enteroviruses, carried out by a team of authors from Laboratory Pirbright, N.J. Knowles being an employee of it, differed from the data on enteroviruses obtained in different countries of the world (including Europe, America, and Asia).

Our data on reproductive properties of porcine enteroviruses in cell cultures of pig fetal kidneys (of English collection and our collection) reveal that all the enterovirus strains without exception were resistant to the effect of chloroform, ether, and trypsin, of media with pH 2.0-11.0; were relatively heat resistant during heating at 56^0C for an hour, with considerable increase of thermal stability in the presence of 1M $MgCl_2$; they formed uniform, pronounced, matted, roundish plaques with smooth contours, and contained RNA.

Investigations of morphology of Konratice strain of Teschen disease and two field strains of porcine enteroviruses revealed accumulation of extracellular virions. The virion diameter equaled to 260-270 Å (26-27 nm), and the capsid diameter comprised 50Å. Electron microscopy studies have enabled to determine their morphological identity with viruses of humans and animals (photograph 1).



Photograph 1. Electron microphotograph of Konratice reference strain of porcine enterovirus of the first serotype. Magnification X 267000.

Following the reports by Knowles N.J. in March 1997 in Internet „Molecular Identification of Porcine Enteroviruses with the Question "Are Teschen-like Viruses True Enteroviruses?" and the report of C.R. Pringle (1999), secretary of ICTV, concerning virus taxonomy on XI International Congress of Virology, Sydney, Australia, 1999, we carried out repeated experiments for study of cytopathogenic properties and thermal resistance of 23 reference strains of porcine enteroviruses from our collection, also including English reference strains of serotypes 1-8 we had obtained in 1970 from Dr. Derbyshire J.B. (Laboratory Pirbright, England); the latter ones, always used in our studies, are similar to the ones used in experiments of Knowles N.J.

The results of the performed experiments are summarized in Tables 2, 3, and 4.

Table 2 shows the results of cytopathic activity of porcine enterovirus reference strains in transferable cell culture line of pig fetal kidney (TPFK) in three consecutive passages without clearly determined inoculative doses of virus strains used in the experiments.

Table 3 shows the results of cytopathic activity of porcine enterovirus reference strains in TPFK cell culture at inoculative dose 100 TCD_{50}.

Table 4 presents the results of thermal resistance of porcine enterovirus reference strains, determined by the method of viral titration in TPFK cell culture, using non-heated (the controls) and heated viruses (1 hour at temperature 56^0C) in the absence and in the presence of 1M $MgCl_2$.

Table 2. Cytopathic activity of porcine enterovirus reference strains in TPFK cell culture using non-controlled inoculative doses

No	Strain name	Sero-type	Origin	Passage I				Passage II				Passage III			
				24	48	72	96	24	48	72	96	24	48	72	96
1	Conratice	1	England	#				#				#			
2	Talfan	1	England	++				+++				+++			
3	Teschena	1	England	+++				+++				#			
4	T-80	2	England	#				+++				#			
5	T-52A	2	England	+++				+++				#			
6	F 59	2	England	++				+++				+++			
7	F 34	3	England	++				+++				+++			
8	F 78	4	England	+++				#				#			
9	F 12	5	England	+++				#				#			
10	F 7	6	England	#				#				#			
11	V 13	8	England	#				#				#			
12	M 2323	10	Ukraine	+++				+++				+++			
13	К-9	11	Ukraine	++				++				#			
14	К-22	12	Ukraine	+++				+++				#			
15	Л-90	13	Ukraine	+++				+++				+++			
16	М-116	14	Ukraine	-	-	++		+	++			+	+++		
17	Ч-73	15	Ukraine	-	+	++		±	++			+	+++		
18	Г-95	16	Ukraine	+	++			++				+++			
19	Б-111	17	Ukraine	++				+++				+++			
20	Ч-184	18	Ukraine	±	+	+++		+	+++			++			
21	Д-229	19	Ukraine	+	++			+	+++			++			
22	И-249	20	Ukraine	+++				#				+++			
23	П-142	21	Ukraine	++				#				+++			
24	В-151	22	Ukraine	#				#				+++			
25	И-393	23	Ukraine	±	+	++		+	++			++			

Note: + - 25% of the cell monolayer are infected by the virus,

++ - 50% of the cell monolayer are infected by the virus,
+++ - 75% of the cell monolayer are infected by the virus,
# - 100% of the cell monolayer are infected by the virus.

Table 3. Cytopathic activity of porcine enterovirus reference strains in TPFK cell culture using inoculative dose 100 TCD_{50}.

No	Strain name	Sero-type	Origin	Term of CPE manifestation, hours				
				24	48	72	96	120
1	Talfan	1	England	+	++	#		
2	T-80	2	England	+	+++	#		
3	F 34	3	England	+	+++	#		
4	F 78	4	England	-	+	++	#	
5	F 12	5	England	+	++	#		
6	F 7	6	England	+	+++	#		
7	V 13	8	England	+	++	#		
8	„Perechinsky 642”	1	Ukraine	+	+++	#		
9	„Bereznyansky 652”	1	Ukraine	+	+++	#		
10	M 2323	10	Ukraine	+	++	+++	#	
11	К-9	11	Ukraine	+	++	#		
12	К-22	12	Ukraine	+	+++	#		
13	Л-90	13	Ukraine	+	++	#		
14	М-116	14	Ukraine	+	#			
15	Ч-73	15	Ukraine	+	+++	#		
16	Г-95	16	Ukraine	+	#			
17	Б-111	17	Ukraine	+	++	#		
18	Ч-184	18	Ukraine	+	#			

Table 4. Thermal resistance of porcine enteroviruses during heating at 56^0C for 1 hour in the absence and in the presence of 1M $MgCl_2$

No	Strain name	Sero-type	Origin	Cell culture	Porcine enterovirus titer. lg TCD_{50}/cm^3		
					Non-heated (control)	Heated without 1 M $MgCl_2$	Heated in the presence of 1 M $MgCl_2$
1	Talfan	1	England	TPFK	9.5±0.25	8.92±0.08	9.17±0.08
2	T 80	2	England	TPFK	9.58±0.08	7.58±0.08	9.08±0.08
3	F 34	3	England	TPFK	9.08±0.08	6.42±0.08	8.92±0.08
4	F 78	4	England	TPFK	8.59±0.08	7.42±0.08	8.42±0.08
5	F 12	5	England	TPFK	8.08±0.08	6.58±0.08	7.42±0.08
6	F 7	6	England	TPFK	9.0±0.15	8.42±0.17	8.92±0.08
7	V-13	8	England	TPFK	8.33±0.17	7.83±0.17	8.33±0.17
8	“ Perechinsky - 642”	1	Ukraine	TPFK	8.58±0.08	7.83±0.17	8.33±0.08
9	“ Bereznyansky 652”	1	Ukraine	TPFK	8.42±0.08	6.42±0.08	8.08±0.08
10	M 2323	10	Ukraine	TPFK	8.83±0.17	8.08±0.08	8.67±0.08
11	К9	11	Ukraine	TPFK	8.33±0.17	7.5±0.29	8.42±0.08
12	К22	12	Ukraine	TPFK	8.42±0.08	7.42±0.08	8.42±0.08
13	Л 90	13	Ukraine	TPFK	8.92±0.08	7.42±0.08	8.58±0.08
14	М 116	14	Ukraine	TPFK	8.58±0.08	7.58±0.08	8.58±0.08
15	Ч 73	15	Ukraine	TPFK	8.42±0.08	7.08±0.08	8.42±0.08
16	Г 95	16	Ukraine	TPFK	9.42±0.08	8.42±0.08	8.92±0.08
17	Б 111	17	Ukraine	TPFK	9.42±0.08	6.08±0.08	8.92±0.08
18	Ч 184	18	Ukraine	TPFK	8.92±0.08	6.08±0.08	8.92±0.08
19	Д 229	19	Ukraine	TPFK	8.92±0.22	8.08±0.08	8.58±0.08
20	И 249	20	Ukraine	TPFK	8.08±0.08	7.42±0.08	7.92±0.08
21	П 142	21	Ukraine	TPFK	8.42±0.08	7.42±0.08	8.08±0.08
22	В 151	22	Ukraine	TPFK	9.42±0.08	8.42±0.08	8.92±0.08
23	И 393	23	Ukraine	TPFK	8.08±0.08	7.92±0.08	8.08±0.08

Note: - M ±m, n=3; $P<0.05$.

The data presented in Tables 2 and 3 evidence that the studied reference strains of porcine enteroviruses, including serotypes 1-8 used for investigations of Knowles N.J., cause cytopathic changes in cell cultures inoculated with either non-controlled viral dose or viral dose 100 TCD_{50}, in a day instead of 4-5 days reported by Knowles N.J.

The results of thermal resistance studies of porcine enteroviruses, presented in Table 4, reveal insignificant decrease of viral titer compared to controls in reference strains heated at 56^0C for 1 hour without 1M $MgCl_2$ while in the presence of 1 M $MgCl_2$ virus titers were almost identical to the titers of control non-heated viral specimens.

Thus, the presented results of long-term experiments beginning from 1969 till now on investigation of porcine enteroviruses (collection of Laboratory Pirbright and our collection), including the experiments carried out in 2008, i.e. following the report by Knowles N.J. and the decision of XI-th International Congress of Virology, confirm that the porcine enterovirus strains we have studied and classified, including English reference strains of porcine enteroviruses of 1-st – 8-th serotypes, obtained from Dr. Derbyshire J. (Laboratory Pirbright, England) in 1970, also used in studies by Knowles N.J., are enteroviruses of Picornaviridae family, Enterovirus genus.

Our data also prove that the distinction of porcine enteroviruses into two genera, Enterovirus и Teschovirus, proposed by Knowles N.J. and approved by XI-th International Congress of Virology, is ungrounded and does not reflect the reality.

Besides, the genus name can not originate from a place Tezen on a Czech-Polish border, where Trefny L. has diagnosed a new porcine disease called enzootic porcine encephalomyelitis in 1930, which was described by Klobauk A. in 1933. Later, this disease appeared in several other countries and acquired different names, including porcine poliomyelitis, Bohemian plaque, Klobauk disease, Talfan disease, Teschen disease, Teschen's disease, viral porcine meningoencephalitis etc.

As is generally known, human poliomyelitis is caused by poliomyelitis virus (the first three serotypes in classification of human enteroviruses); the mentioned classification also includes Coxsackie and ECHO viruses, among them Coxsackie B5 of serological relation with porcine vesicular disease (Brown, Wild, 1974). Nevertheless, Coxsackie B5 virus is not excluded from Enterovirus genus.
Besides, the habitat of porcine enteroviruses of all serotypes, including serotype 1 and 2, being the causative agents of poliencephalomyelitis in pigs, is the intestine, where (in mucous coat epithelium) their reproduction occurs. Following reproduction in intestinal epithelium, enteroviruses of certain serotypes penetrate

into other organs and tissues, where they cause corresponding pathological changes.

Literature

1. Genetic markers of enteroviruses isolated from lungs of pigs suffering from pneumonia / V.P. Romanenko, I.M. Pinchuk. // Bulletin of Agricultural Science, 1982, No 10, p. 47-49.

2. Classification of porcine enteroviruses / Romanenko V.F., Pruss O.G., Bokun A.A., Babych N.V., Polevik E. N., Pinchuk I.N. // Bulletin of Agricultural Science. – K., 1993. – No1. – P. 94-101.

3. Taxonomy of Porcine Enteroviruses / Romanenko V.F., Polevik E. N., Pruss O.G., Babych N.V., Bokun A.A., Pinchuk I.N. // Veterinary Medicine. – M., 1993. – No 5. – P. 26-29.

4. Population analysis of porcine enteroviruses isolated in farms of Ukraine / Romanenko V.P., Pinchuk I.M. // Microbiological Journal, 1994, No 5, vol. 56, p. 102.

5. Human enterovirus infections / M. K. Voroshilova. – Moscow. – Medicine.- 1979.- 360 p.

6. Poliomyelitis / Chumakov M.P., Prisman I.M., Zatsepin G.S.- Moscow.- Medgiz. -1953.

7. Immunology, epidemiology, and prophylaxis of poliomyelitis and similar diseases / Voroshilova M. K. .- Moscow.- Medicine. – 1966.-435 p.

8. Betts A.O.Studies on entheroviruses of the pig II/ The effect of temperature ultra-violet light and other on the T 80 strain of a swine polioencephalomyelitis virus // Res. Vet. Sci.- 1960.-N1.-P.65-71.

9. Lamont P.H. Studies on cytopathogenic agents recovered from the faeces of swine: Diss. Pn. D. − Thesis/Univ. Cambridge.−England, 1960. - 24 p.

10. Indication of multiplication of porcine enterovirus within the intracellular canalicular system/Bjőrkman, Sibalin // Virology, 1960.- V11.- N.2.- P.513-518.

11. Derzeitiger Stand unserer Konntnisse űber die ECSO-viren/ Pette J. // Berl.u.Műnch.tierärztl. Wochenschr.,1966.- V79.- N1.- P.6-10.

12. Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues / Enders J.F., Waller T.H., Robbins F.C. // Science, 1949.-V.109.-P.85-87.

13. Betts A.O., Lamont P.H., Kelly D.F. Porcine entheroviruses other than the virus of Teschen disease/Ann. New York Acad.Sci., 1962.- 101.- P.428-435.

14. Betts A.O., Jennings A.R. Kelly D.F. Studies on enteroviruses of pigs I. The recovery in tissue culture of two related strains of swine polioencephalomyelitis virus from the tonsils of “normal” pigs // Res. Vet. Sci.- 1960.- V.1.-N.1.- P. 157-164.

15. Zoletto R. Characteristiene differeziali degli enterovirus suini.Atti Soc.Ital.sci vet.-1964.-18.-P. 694-695.

16. Zoletto R. Differential characteristics of swine enteroviruses//Vet. Ital.-1965.-V.16.P.13-20.

17. Kadoi K., Kabori S., Morimoto T. Studies of swine enteroviruses: Japanese 6th serotype and relationship between heat susceptibility and cytopathic effects // Jap. J. Microbiol.– 1970.– V.14.N2– P.111-121.

18. Pleva, Mesaros E. Cytopathological changes produced in tissue cultures by enteroviruses I pug kidney tissue cultures infected with enteroviruses isolated from pigs//Docum. Vet.-1968.-N.6.P.187-192.

19. Romanenko V.P. Porcine enteroviruses, causative agents of enzootic encephalomyelitis and gastroenteritis of pigs: Theses.…Doctor of Veterinary Science: 16.00.03. – Ch., 1977.

20. Alexander T. J. L., Betts A.O. Futder studies on porcine enteroviruses isolated at Cambridge // Serological grouping // Res. Vet.Sci.-1967.-V8, N3.-P.330-337.

21. Dunne H.W., Wang T.J., Ammerman E.H. Classification of North American porcine enteroviruses: a comparison with Europian and Japanese strains // Infect. Immunol. – 1971. – V.4, №5.–P.619-631.

22. Dunne H.W., Wang T.J. Immune response to porcine entheroviruses: effect of antigenic variations on classification // J. Am. Vet. Med. Assn.-1972.-160.-4.-613-622.

23. Rasmussen P.G. A study of enterovirus strains in Danish pigs.III. Division of enterovirus strains into two groups based cytopathogenic effect, thermoresistanse (50^0C) and pH stability (pH 2.2)// Nord. Vet.Med. – 1969. – V21. – №4. – P.117-187.

24. Christov S. Isolation and serological grouping of swine entheroviruses.− Polskie Arch. Vet. 1966, v.9.,N.3,p.445-449.

25. Knowles, N.J., Buckley, L.S. and Pereira, H.G. Classification of porcine enteroviruses by antigenic analysis and cytopathic effects in tissue culture: description of 3 new serotypes // *Archives of Virology*.– 1979 – № 62.– P. 201-208.

26. Sero- and CPE- types at porcine enteroviruses isolated from hearthy and diarrheal pigs : possible association of CPE type II with diarrhea / Honda E., Hattori I., Oohara Y et al // Japanese Journal of Vet. Science. - 1990.-V.52, N1.-P.85-90.

27. Typisierung von 17 porzinen enterovirusisolaten aus polioenzephalomyelitisfallen der Jahre 1983-1991 / Witte K. H., Auerbach J., Loss K.U. et al. // Deutsche tierarzliche.-Wochenschrift.- 1994.- V. 101, N.12.-P. 482-484.

28. Laboratory Identification of Porcine Enterovirus / Knowles N.J.- International symposiumum on porcine picornaviridae, Greifswald, Germany, 2-3 May 1994.

29. Pringle CR (1999) Virus Taxonomy – 1999. Arch. Virol 144: 421-429.

30. Thermal resistance of porcine enteroviruses: Proceedings of the I-st International Congress of Specialists in Veterinary Medicine, (Kyiv, 6-10 October 2008)/ National Agricultural University, 2008.

31. Mefianu (1979) La malalie de Teschen, Bulletin Mensuel de la sosiete Veterinaire Pratique de la France, 63, 503-517.

32. Klobauk A. – Kotazce actiologie tau zu tesinske nemoci – Encephalomyelitis enzootica suum (obrna repru) Predblizne sdeleni. Zverolekarsky Rozpravy, 1933, 7, 85 (abstracted in Vet. Bull.), 1934, 4, 467.

33. Trefny L. – Hromadna onomacnevi vepru na Tesinsku. Zwerol Obzor, 1930, 23, 235-236.